A Meta-Analytic Summary of NIT Applications in NASH Development

Liver Forum 'Disease Assessment Strategies to Accelerate Drug Development '
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Professor Quentin M. Anstee PhD, FRCP
Professor of Experimental Hepatology & Honorary Consultant Hepatologist,
Translational & Clinical Research Institute,
Newcastle University, UK.

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Disclosure Slide

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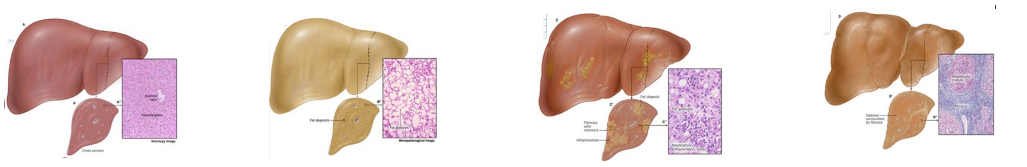
Non-Invasive Tests & Biomarkers Across the Spectrum of NAFLD



Normal Liver	Steatosis (NAFL)	Steatohepatitis (NASH)	Fibrosis & Cirrhosis
"Wet" Biomarkers	Fatty Liver Index (FLI)	CK-18 OWL™ Metabolomics NIS4™ SomaSignal™	"Simple" Scores (FIB4, NFS) Direct Collagen Biomarkers (ELF Test™, PRO-C3™) SomaSignal™
"Dry" (Imaging) Biomarkers	Ultrasound FibroScan™ (CAP) MR-PDFF	FAST Score™ MAST Score MR Liver MultiScan™ (cT1)	FibroScan™ (VCTE) Agile 3+/4™ Ultrasound: SSI, ARFI MR Elastography

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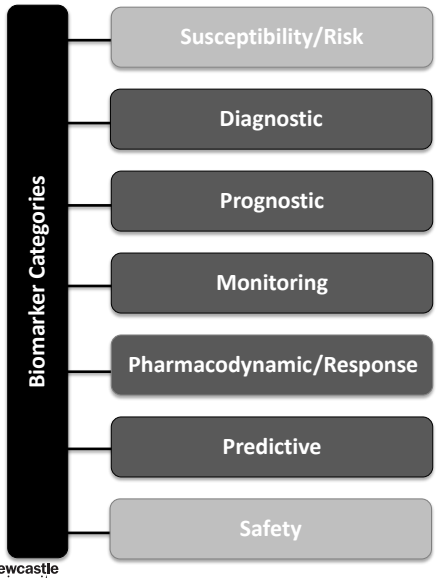
NIT Applications in NASH Drug Development



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FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource https://www.ncbi.nlm.nih.gov/books/NBK338448/

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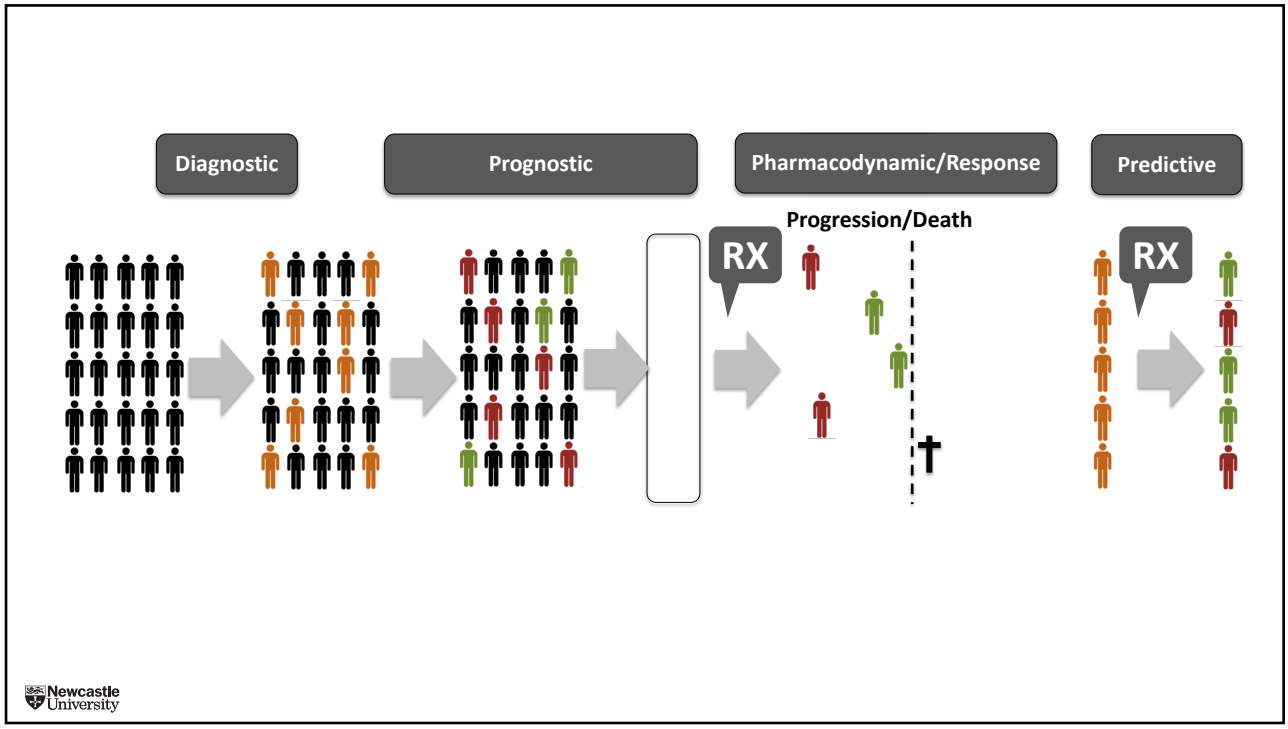

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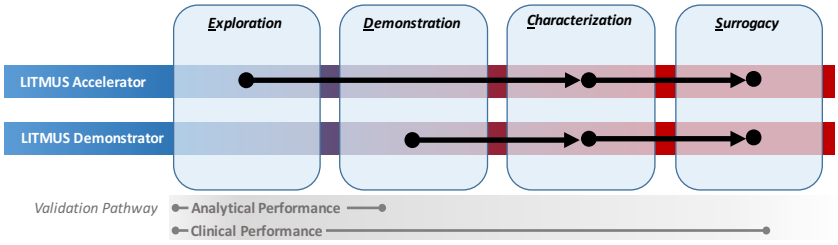
A Systematic Approach to Biomarker Evaluation



Authoritative Systematic Reviews & Individual Patient Data Meta-Analyses → Biomarker Measurement & Performance Assessment in the Retrospective/Extant 'LITMUS Metacohort' (1a) → Biomarker Measurement & Performance Assessment in the Prospective 'LITMUS Study Cohort' (1b)

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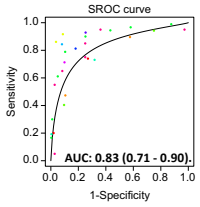
Performance of Enhanced Liver Fibrosis (ELF®) Test in NAFLD

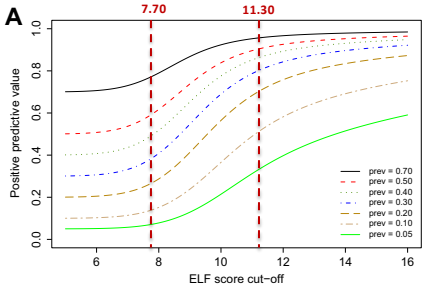
Meta-analysis of 11 studies including 4,452 NAFLD patients

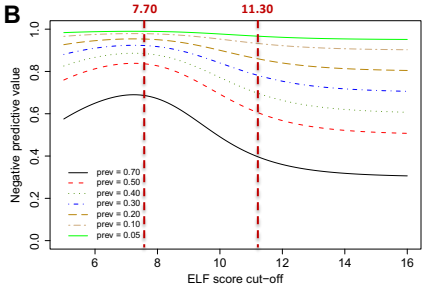
ELF is a combination of 3 direct markers of fibrosis:

- **Procollagen III N-terminal peptide (PIIINP)**
- **Hyaluronic acid (HA)**
- **Tissue inhibitor of metaloproteinase 1 (TIMP1)**

Cut-off	Se (95%CI)	Sp
7.70	0.93 (0.82-0.98)	0.34 (0.13-0.65)
9.80	0.65 (0.49-0.77)	0.86 (0.77-0.92)
10.51	0.51 (0.31-0.70)	0.93 (0.85-0.96)
11.30	0.36 (0.15-0.63)	0.96 (0.90-0.99)







Cut-off	Se	Sp	Prev	PPV	NPV
9.80	0.65	0.86	**0.05**	0.19	0.98
			0.10	0.33	0.96
			0.20	0.53	0.91
			0.30	0.66	0.85
			0.40	0.75	0.78
11.30	0.35	0.96	**0.05**	0.33	0.97
			0.10	0.51	0.93
			0.20	0.70	0.86
			0.30	0.80	0.78
			0.40	0.86	0.69

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1. Vali Y et al. Enhanced liver fibrosis test for the non-invasive diagnosis of fibrosis in patients with NAFLD: A systematic review and meta-analysis. J Hepatol 2020;73:252-262.

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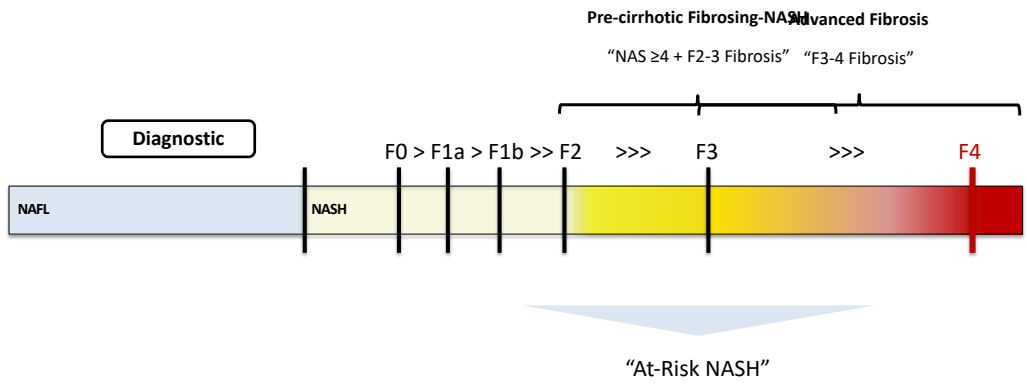
Defining the Target Condition: 'At-Risk' NASH & 'Advanced Fibrosis'



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Thresholds to Control Screen Failure Rates for Pre-Screening in Clinical Trials

LITMUS Metacohort: To control biopsy screen failure rate to <1:3 for 'At-Risk NASH' (NAS ≥4, F≥2) at a 35% prevalence

Marker	Sensitivity	Specificity	Number of positive patients undergoing biopsy (Per 100)	Number of eligible patients found (Per 100)	Number needed to test
SomaSignal	0.67	0.82	35	24	4
ADAPT	0.47	0.88	24	16	6
MACK-3	0.41	0.89	21	14	7
PRO-C3	0.33	0.92	17	11	9
FIBC3	0.28	0.93	14	10	10
CK-18 M30	0.25	0.93	13	9	11
PRO-C6	0.18	0.96	9	6	16
PRO-C4	0.12	0.97	6	4	23
CK-18 M65	0.12	0.97	6	4	24
No marker	-	-	100	35	-

*** No acceptable performance threshold was found for APRI, NFS, FIB-4 or ELF.**

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1. Vali, Lee et al. 'Comparative diagnostic accuracy of blood-based biomarkers for staging at-risk NASH in NAFLD: first results of the LITMUS project', under review, 2022

LITMUS

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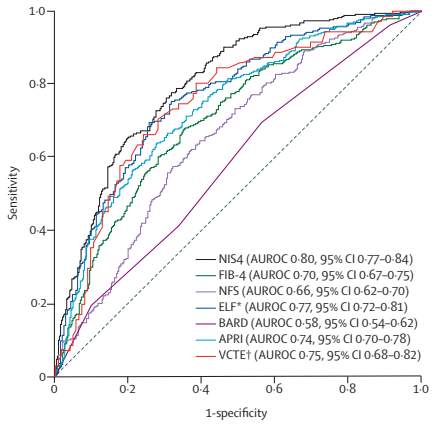
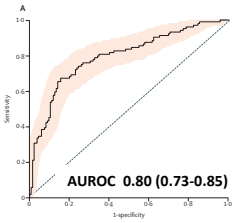
Performance of the NIS4 Score for 'At-Risk' NASH (NAS≥4 + F≥2)

NIS4 Score vs. Other Biomarkers

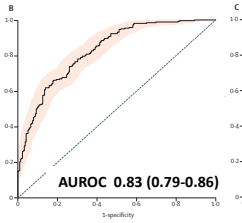


Four components of NIS4:
miR-34a, Alpha-2 Macroglobulin, HbA1c & YKL-40

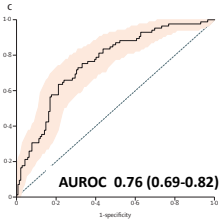
Training
GOLDEN (239 pts)



Validation #1
RESOLVE-IT-DIAG (475 pts)



Validation #2
Angers Hepatology (227 pts)



	Number of patients with both comparator test and NIS4 data	Number of patients with condition	Comparator test AUROC (95% CI)	NIS4 reference AUROC (95% CI)	p value
At-risk NASH (NAS ≥4 and fibrosis stage ≥2)					
NIS4	702	345	..	0·80 (0·77–0·84)	..
FIB-4	694	341	0·70 (0·67–0·75)	0·81 (0·77–0·84)	<0·0001
NFS	694	341	0·66 (0·62–0·70)	0·81 (0·77–0·84)	<0·0001
ELF*	474	259	0·77 (0·72–0·81)	0·83 (0·79–0·86)	0·0067
BARD	702	345	0·58 (0·54–0·62)	0·80 (0·77–0·84)	<0·0001
APRI	694	341	0·74 (0·70–0·78)	0·81 (0·77–0·84)	0·0010
VCTE†	196	71	0·75 (0·68–0·82)	0·76 (0·69–0·82)	0·92

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Harrison et al, Lancet Gastro Hepatol, 2020.

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NIS4 for Identification of 'At-Risk' NASH

Analysis of NAFLD cases from the NIMBLE Phase 1 (NASH-CRN) Cohort

	AUROC NIS-4	AUROC ALT	P-value vs unit line	P-value vs ALT
NASH	0.832	0.678	<0.001	<0.001
NAS ≥4	0.815	0.726	<0.001	<0.001
	AUROC NIS-4	**AUROC FIB-4**	**P-value vs unit line**	**P-value vs FIB-4**
≥Stage 2	0.874	0.796	<0.001	<0.001
≥Stage 3	0.788	0.793	<0.001	0.6
Stage 4	0.725	0.815	<0.001	1

AUROC NIS-4	P-value vs unit line	AUROC FIB-4	P-value vs FIB-4
0.815	<0.001	0.762	<0.001

Youden ≥0.6 (sensitivity 78.1%; specificity 73.6%)

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1. Sanyal AJ, et al. AASLD 2021. #LO1

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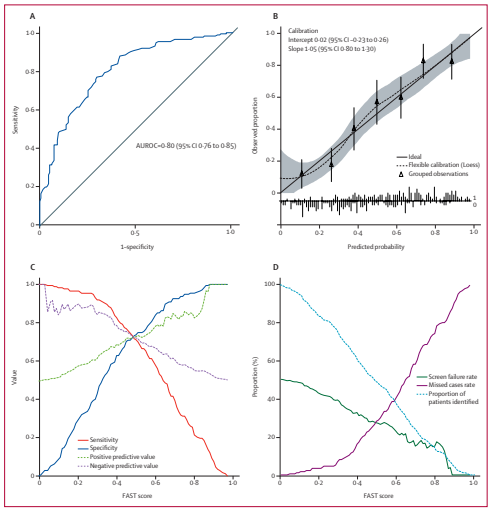
Fibroscan-AST (FAST) Score for 'At-Risk' NASH ≥F2

- Target condition: **NASH+Fibrosis (NAS≥4 + F≥2)** for clinical trial enrolment.
- FAST components VCTE, CAP and AST

- **Derivation cohort** (n=350, 50% NAS≥4 + F≥2)
  - AUROC 0.80 (0·76-0·85)
- **International validation cohorts** (n=1026, 27% NAS≥4 + F≥2)
  - AUROC 0.85 (0·83–0·87)

Cut-off	≤0.35	≥0.67
Se / Sp	Se=0.89 / Sp=0.64	Se=0.92 / Sp=0.49
PPV / NPV	NPV=0.94	PPV=0.69
Indeterminates	30%	



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1. Newsome PN et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. Lancet Gastroenterol Hepatol 2020;5:362-373.

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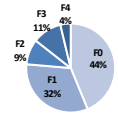
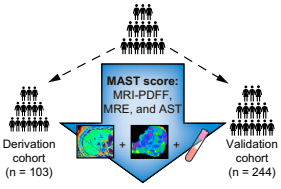
MAST (MRE, PDFF & AST) Score to Detect 'At-Risk' NASH ≥F2

MAST developed in a US cohort for 347 histologically characterised NAFLD patients (cases preselected with PDFF >5% and VCTE >7kPa/MRE >2.5kPa)

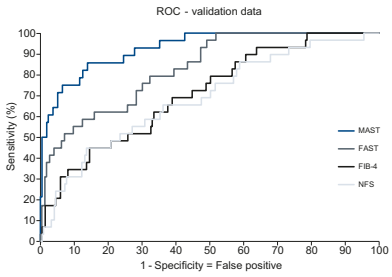
Variables
MRE, MRI-PDFF, ALT, AST, AST/ALT ratio, Alb, Plt, T2DM, Sex, Age & BMI



13% NAS ≥4 + F≥2



MAST Score = -12.17 + 7.07 log MRE + 0.037 PDFF + 3.55 log AST



Score	Sample	ROC area	Sensitivity	Specificity	PPV	NPV
MAST	Derivation	0.858	94.4%	72.9%	42.5%	98.4%
MAST	Validation	0.929	89.3%	73.1%	30.1%	98.1%
FAST	Validation	0.868	93.1%	64.1%	25.0%	98.6%
NAFLD (NFS)	Derivation	0.748	100.0%	52.9%	30.5%	100.0%
NAFLD (NFS)	Validation	0.689	58.6%	66.6%	18.7%	92.5%
Fib-4	Derivation	0.891	88.9%	74.7%	42.1%	97.0%
Fib-4	Validation	0.711	20.7%	95.5%	37.5%	90.2%

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1. Noureddin M, et al. MRI-based (MAST) score accurately identifies patients with NASH and significant fibrosis. J Hepatol 2022;76:781-787.

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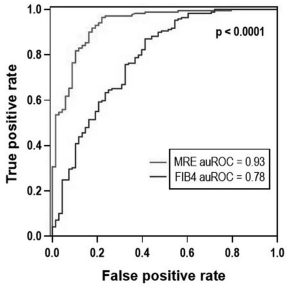
MRE combined with FIB-4 (MEFIB) to Detect 'At-Risk' NASH ≥F2

- Target condition: **NASH+Fibrosis (F≥2)** for clinical trial enrolment.
- MEFIB components: **FIB-4 ≥1.6 plus MRE ≥3.3 kPa**

- **Derivation cohort** (n=238, 29% F≥2)
  - AUROC 0.90 (0.85 to 0.95), PPV 97.1% NPV 83.2%
- **International validation cohorts** (n=222, 61% F≥2)
  - AUROC 0.84 (0.78 to 0.89), PPV 91.0% NPV 59.4%



	Discovery Cohort					Validation Cohort				
Model	AUROC (95% CI)	OR (95% CI)	P value*	PPV‡	NPV	AUROC (95% CI)	OR (95% CI)	P value	PPV‡	NPV
MRE ≥3.3 kPa†	0.87 (0.81 to 0.92)	MRE: 71.55 (28.73 to 178.18)	Ref	86.9	91.5	0.79 (0.74 to 0.85)	MRE: 14.74 (7.60 to 28.57)	Ref	84.6	72.8
FIB-4 ≥1.6†	0.72 (0.66 to 0.78)	FIB-4: 8.23 (4.32 to 15.65)	**0.0002**	61.5	83.7	0.73 (0.68 to 0.79)	FIB-4: 8.31 (4.35 to 15.88)	0.0792	84.6	60.2
MRE ≥3.3 kPa + FIB-4 ≥1.6	0.90 (0.85 to 0.95)	MRE: 56.41 (21.80 to 145.94) FIB-4: 5.16 (2.04 to 13.06)	**0.0184**	97.1	83.2	0.84 (0.78 to 0.89)	MRE: 8.96 (4.41 to 18.22) FIB-4: 3.57 (1.69 to 7.51)	**0.0026**	91.0	59.4
		MEFIB misclassified: 35/238 (15%)					**MEFIB misclassified: 62/222 (28%)**			

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1. Jung J, et al. MRE combined with FIB-4 (MEFIB) index in detection of candidates for pharmacological treatment of NASH-related fibrosis. Gut 2021;70:1946-1953.

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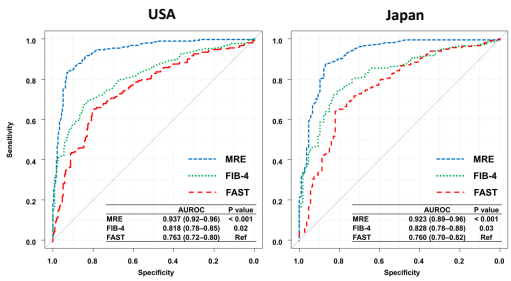
MRE plus FIB-4 (MEFIB) versus FAST to Detect 'At-Risk' NASH ≥F2

Comparison of MEFIB vs. FAST in 234 US cases + 314 Japanese cases to detect Fibrosis ≥F2

- "Rule in": **FIB-4 ≥1.6 plus MRE ≥3.3 kPa** or **FAST ≥ 0.67**
- "Rule out": **FIB-4 <1.6 plus MRE <3.3 kPa** or **FAST < 0.35**



	AUROC	Rule In			Indeterm.	Rule Out		
		Sens	Spec	PPV		Sens	Spec	NPV
MEFIB	**0.899 (0.86-0.94)**	0.69	0.94	95.6%	23.9%	0.94	0.73	85.6%
FAST	0.724 (0.67-0.78)	0.28	0.93	89.2%	42.4%	0.76	0.63	57.8%

* Data shown from Japanese cohort (similar results in USA cohort and combined cohorts)

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1. Tamaki N, et al. MRE plus FIB-4 (MEFIB) versus FAST in detection of candidates for pharmacological treatment of NASH-related fibrosis. Hepatology 2021.

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ELF and PRO-C3 for Identification of 'Significant' & 'Advanced' Fibrosis

Analysis of NAFLD cases from the NIMBLE Phase 1 (NASH-CRN) Cohort

PRO-C3

	≥Stage 2	Stage 3 or 4	Stage 4
AUROC (PRO-C3)	0.809	0.764	0.728
AUROC (FIB-4)	0.799	0.79	0.81
Is AUROC >0.7 and superior to 0.5?	<0.001	<0.001	<0.001
Is AUROC superior to FIB-4?	0.27	0.9	1.0
Performance statistics for PRO-C3			
Youden index cutoff	≥17.6	≥18.8	≥21.1
Sensitivity	69.8	71.4	66.2
Specificity	81.0	71.4	68.5

ELF

	≥Stage 2	Stage 3 or 4	Stage 4
AUROC (ELF test)	0.828	0.835	0.855
AUROC (FIB-4)	0.798	0.789	0.81
Is AUROC >0.7 and superior to 0.5?	<0.001	<0.001	<0.001
Is AUROC superior to FIB-4?	0.01	<0.001	<0.001
Performance statistics for ELF test			
Youden index cutoff	9.5	9.6	10.1
Sensitivity	71.8	80.8	82.1
Specificity	81.5	70.2	73.3

ELF performance improved progressively for diagnosis of progressively more advanced fibrosis

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1. Sanyal AJ, et al. AASLD 2021. #LO1

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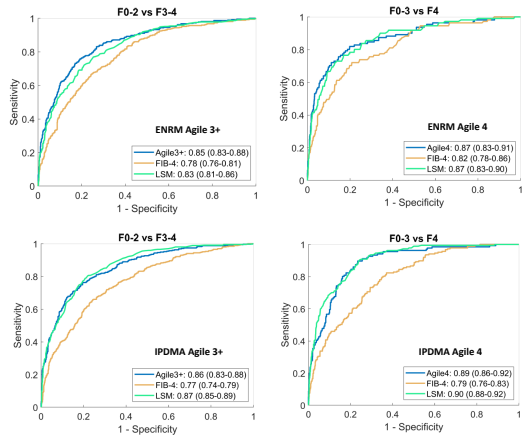
Agile 3+ & Agile 4 for Advanced Fibrosis & Cirrhosis

Analysis of two large international NAFLD cohorts: LITMUS Metacohort (n=1271) & IPD Meta-analysis (n=1292)



Agile 3+/4 are Composite scores combining VCTE with ALT/AST, Plt, T2DM, Gender, Age

		Advanced Fibrosis (F3-4)			Cirrhosis (F4)		
		LSM	FIB4	AGILE 3+	LSM	FIB4	AGILE 4
European NAFLD Registry Metacohort	AUROC (95% CI)	0.83 (0.81-0.86)	0.78 (0.76-0.81)	0.85 (0.83-0.88)	0.87 (0.83-0.90)	0.82 (0.78-0.86)	0.87 (0.83-0.91)
	Se/Sp	0.79/0.72	0.30/0.68	0.68/0.70	0.77/0.82	0.50/0.61	0.59/0.85
	PPV/NPV	0.54/0.89	0.66/0.85	0.66/0.92	0.28/0.97	0.32/0.98	0.50/0.97
	Un-classified, %	0.0	29.8	15.5	0.0	29.8	10.4
	Proportion with target condition, %	29.3			8.7		
IPDMA Cohort	AUROC (95% CI)	0.87 (0.85-0.89)	0.77 (0.74-0.79)	0.86 (0.83-0.88)	0.90 (0.88-0.92)	0.79 (0.76-0.83)	0.89 (0.86-0.92)
	Se/Sp	0.75/0.81	0.46/0.94	0.71/0.90	0.71/0.88	0.62/0.89	0.46/0.97
	PPV/NPV	0.62/0.89	0.73/0.84	0.71/0.90	0.42/0.96	0.38/0.96	0.62/0.95
	Un-classified, %	0.0	26.4	12.2	0.0	26.4	8.4
	Proportion with target condition, %	28.6			11.4		

Thresholds: LSM F3+ ≥9.7 kPa, F4 ≥13.6 kPa; **FIB4** F0-2 <1.3 (<2.0 if age 65+), F3-4 >2.67; **Agile 3+** F0-2 <0.451, F3-4 ≥0.679; **Agile 4** F0-3 <0.251, F4 ≥0.565.

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1. Hardy, Mozes et al. Performance of Agile 3+ and Agile 4 Fibroscan-based Tests for Advanced Fibrosis and Cirrhosis in International Cohorts Comprising over 2,500 Histologically Characterised NAFLD Patients. AASLD dTLM 2021.

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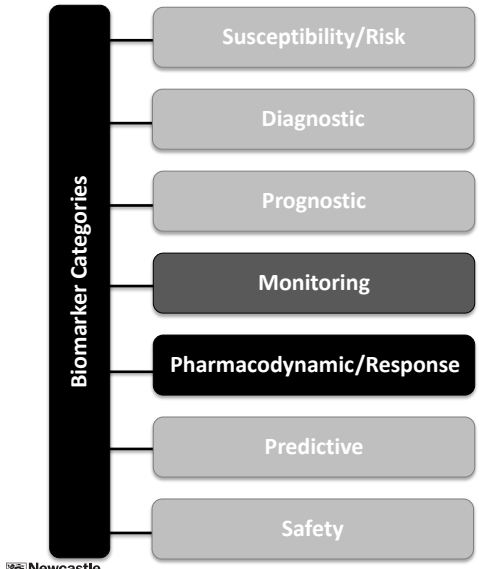
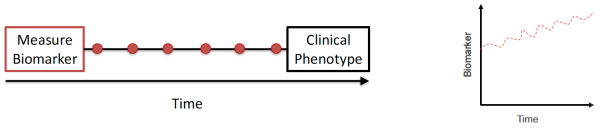
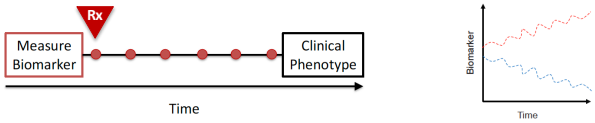
Biomarker Context of Use



Monitoring



Pharmacodynamic /Therapeutic Response



Change in biomarker (continuous, ordinal or categorical) must correlate with a definite change in disease state that relates to outcome.

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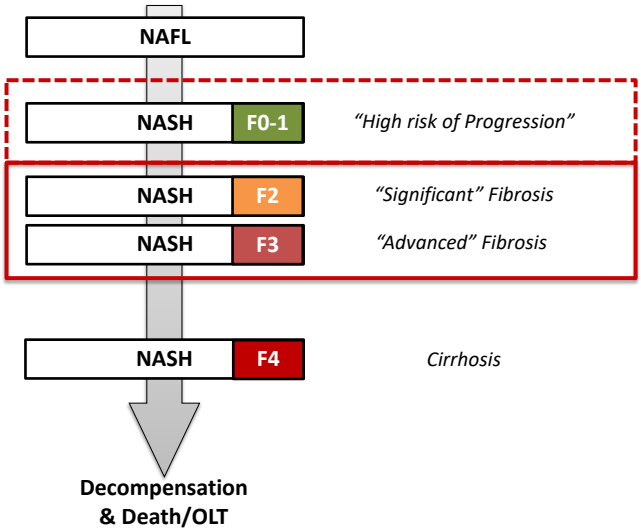
Assessment of Efficacy – Pre-cirrhotic NASH



'Likely Accepted' Surrogates

Histology Endpoints for NASH Trials:

- **Reversal of Steatohepatitis, with no worsening of fibrosis.**
  - Minimum 2-point improvement in NAS (with ≥1 point improvement in ≥1 category)
  - Resolution of NASH (with Ballooning 0 & Inflammation 0-1**)**
- **Improvement of Fibrosis, with no worsening of NASH.**
  - Minimum 1-point improvement of Fibrosis Stage

'Generally Accepted' Surrogate

'Clinically Meaningful' Outcomes

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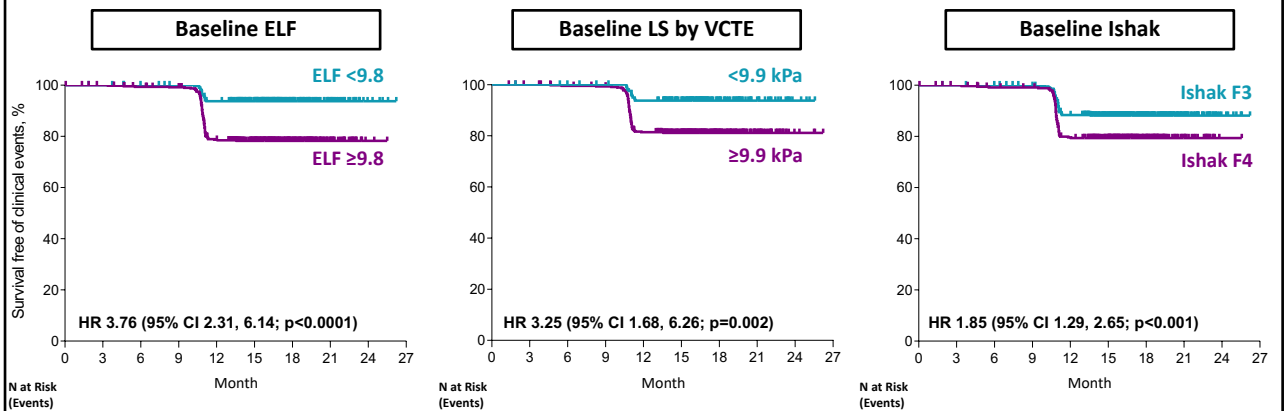
Evidence to Support Fibrosis Biomarkers as Surrogate Endpoints



N at Risk (Events)											
ELF <9.8	313 (0)	311 (0)	307 (0)	304 (0)	283 (19)	214 (19)	88 (19)	33 (19)	6 (19)	0 (19)	
ELF ≥9.8	489 (0)	485 (0)	477 (3)	473 (4)	370 (101)	292 (103)	133 (103)	41 (103)	2 (103)	0 (103)	

N at Risk (Events)											
<9.9 kPa	166 (0)	165 (0)	163 (0)	161 (0)	149 (10)	115 (10)	54 (10)	19 (10)	4 (10)	0 (10)	
≥9.9 kPa	454 (0)	450 (1)	442 (2)	439 (3)	355 (82)	280 (83)	118 (83)	45 (83)	4 (83)	0 (83)	

N at Risk (Events)											
Ishak 3	449 (0)	447 (0)	445 (0)	440 (1)	385 (51)	298 (52)	131 (52)	50 (52)	7 (52)	0 (52)	
Ishak 4	353 (0)	349 (0)	339 (3)	337 (3)	268 (69)	208 (70)	90 (70)	24 (70)	1 (70)	0 (70)	

Baseline ELF (c-statistic 0.68) and LS by VCTE (0.71) more accurate for disease progression than fibrosis stage (0.58)

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STELLAR3: Anstee et al, AASLD 2019.

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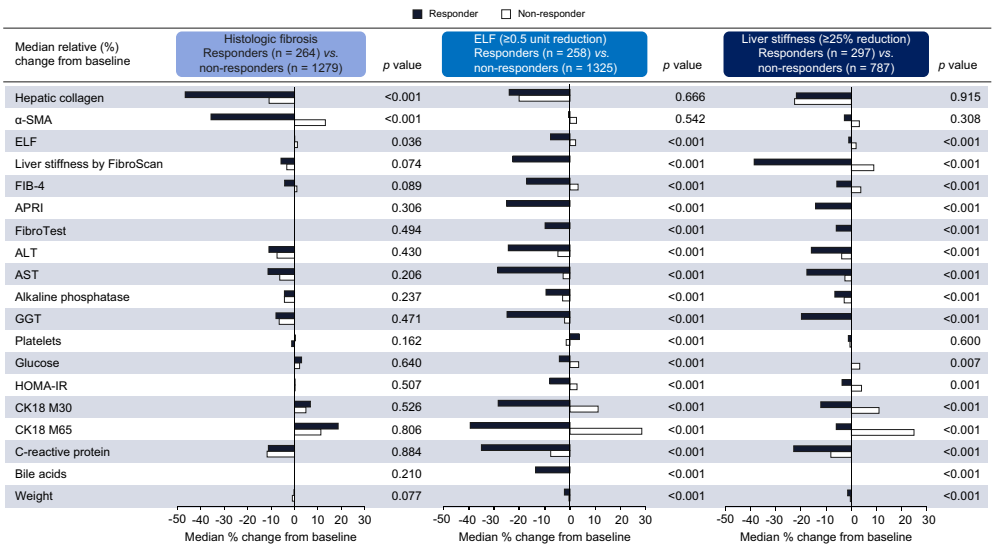
Direction of Change in NITs Appear More Consistent



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All data are median relative change at W48 from BL.

1. Harrison SA, et al. Selonsertib for patients with bridging fibrosis or compensated cirrhosis due to NASH: Results from randomized phase III STELLAR trials. J Hepatol 2020;73:26-39.

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Meta-Thoughts on Response Biomarkers in NASH Drug Development

1. Rapidly attaining a NIT 'response threshold' is not a guarantee of early efficacy.

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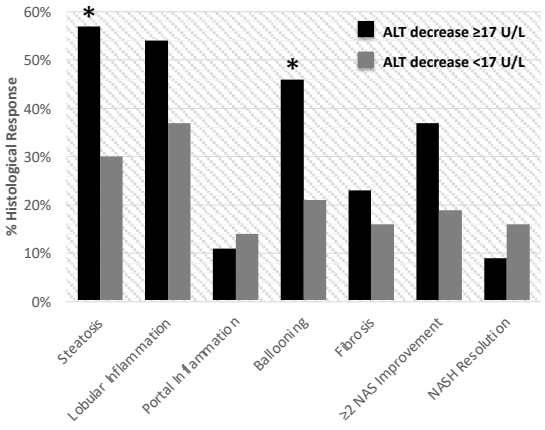
Fall in ALT Correlates with Histological Improvement

Data from subgroup analysis of FLINT Trial at 72-Weeks (n = 78)



	OR (95%CI)	Sens	Spec	PPV	NPV
Steatosis	3.1 (1.2-7.8)	0.61	0.67	0.57	0.70
Lobular Inflammation	2.0 (0.8-5.0)	0.54	0.63	0.54	0.63
Portal Inflammation	0.8 (0.2-3.1)	0.40	0.54	0.11	0.86
Ballooning	3.2 (1.2-8.6)	0.64	0.64	0.46	0.79
Fibrosis	1.5 (0.5-4.7)	0.53	0.57	0.23	0.84
≥2 NAS Improvement w/o fibrosis worsening	2.6 (0.9-7.2)	0.62	0.61	0.37	0.81
NASH Resolution	0.5 (0.1-2.0)	0.30	0.53	0.09	0.84

Predictive Value of ALT Reduction ≥ Threshold 17 IU/L

Newcastle University

1. Loomba et al. Multicenter Validation of Association Between Decline in MRI-PDFF and Histologic Response in NASH. Hepatology 2020;72:1219-1229.

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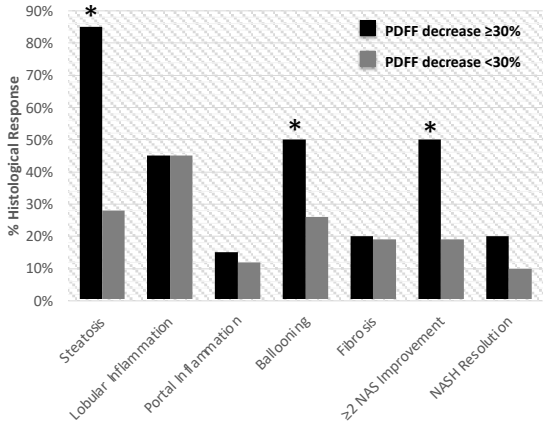
Fall in MRI-PDFF Correlates with Histological Improvement

Data from subgroup analysis of FLINT Trial at 72-Weeks (n = 78)



	OR (95%CI)	Sens	Spec	PPV	NPV
Steatosis	14.9 (3.8-57.7)	0.52	0.93	0.85	0.72
Lobular Inflammation	1.0 (0.4-2.8)	0.26	0.74	0.45	0.55
Portal Inflammation	1.3 (0.3-5.5)	0.30	0.75	0.15	0.88
Ballooning	2.9 (1.0-8.2)	0.40	0.81	0.50	0.74
Fibrosis	1.1 (0.3-3.8)	0.27	0.75	0.20	0.81
≥2 NAS Improvement w/o fibrosis worsening	4.3 (1.4-12.8)	0.48	0.82	0.50	0.81
NASH Resolution	2.3 (0.5-8.6)	0.40	0.76	0.20	0.9

Predictive Value of Relative PDFF Reduction ≥ 30%

Newcastle University

1. Loomba et al. Multicenter Validation of Association Between Decline in MRI-PDFF and Histologic Response in NASH. Hepatology 2020;72:1219-1229.

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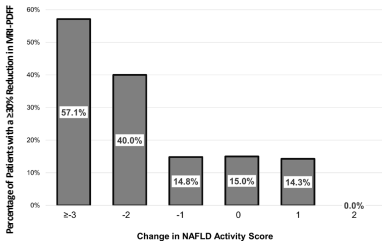
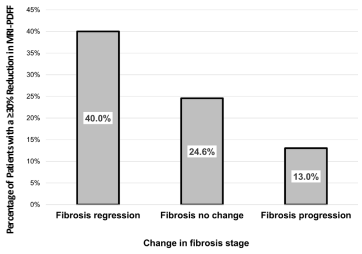
Fall in MRI-PDFF Correlates with Histological Improvement

Analysis of 100 NAFLD cases undergoing paired liver biopsy and MRI-PDFF 1.4 (0.6–2.9) years apart.





Histological response (≥1 point/stage decrease)	Relative decline in MRI-PDFF ≥30% (n=25), %	Relative decline in MRI-PDFF <30% (n=75), %	≥30% vs <30% relative decline in MRI-PDFF OR	95% CI	P value
Steatosis	64	32	3.78	1.5 to 9.8	0.006
Lobular inflammation	44	24	2.49	0.9 to 6.4	0.06
Ballooning	56	41	1.81	0.7 to 4.5	0.2
NAS response*	60	24	4.75	1.8 to 12	0.001
Fibrosis†	50	21	3.75	1.2 to 12	0.03

MRI-PDFF response (≥30%) was an independent predictor of fibrosis regression with an adjusted OR of 6.46 (95% CI 1.1 to 37.0, p=0.04).

Newcastle University

1. Tamaki et al. Clinical utility of 30% relative decline in MRI-PDFF in predicting fibrosis regression in non-alcoholic fatty liver disease. 2022;71:983-990.

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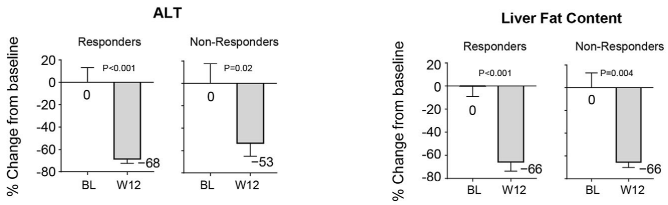
Dissociation of NIT Response Thresholds and Histology in Short-Term Trials

Comparison of changes in ALT & MRI-PDFF in patients with/without histological NAS/Fibrosis improvement



Histological Improvement: ≥2 NAS improvement w/o fibrosis worsening OR ≥1 fibrosis improvement w/o NASH worsening

In short duration studies improvement in ALT and/or PDFF may not correlate with meaningful change in disease state.

Newcastle University

1. Harrison et al. NGM282 Improves Liver Fibrosis and Histology in 12 Weeks in Patients With Nonalcoholic Steatohepatitis. Hepatology 2020;71:1198-1212.

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Meta-Thoughts on Response Biomarkers in NASH Drug Development

1. **Rapidly attaining a NIT 'response threshold' is not a guarantee of early efficacy.**
2. **Interpret changes in 'Simple Panels' (APRI, FIB4, NFS) with caution.**

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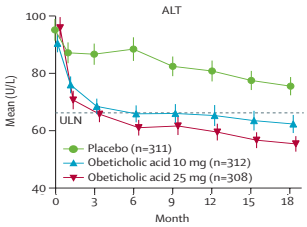
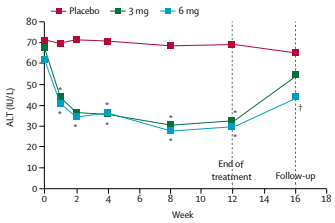
Rapid Changes From Baseline in ALT and AST

Rapid falls in ALT and AST often occur within the first month of therapy – and then plateau through Week 24+

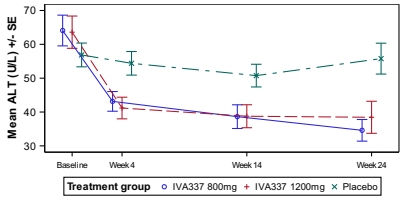
OCA (REGENERATE)



Aldafermin



Lanifibranor (NATIVE)



Drops in 'Simple Panels' (FIB4, APRI, etc) are likely to be driven by this process, not fibrosis regression.

1. Harrison et al. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391:1174-1185. 2. Younossi et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. Lancet 2019;394:2184-2196. 3. Francque et al. A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH. N Engl J Med 2021;385:1547-1558.

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Meta-Thoughts on Response Biomarkers in NASH Drug Development

1. Rapidly attaining a NIT 'response threshold' is not a guarantee of early efficacy.
2. **Interpret changes in 'Simple Panels' (APRI, FIB4, NFS) with caution.**
3. **In the absence of a single 'gold standard' biomarker, undertake a wholistic assessment of biomarker response and demonstrate change *at the patient level*.**

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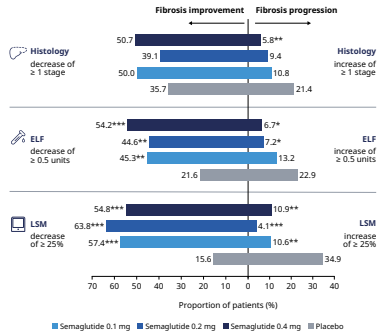
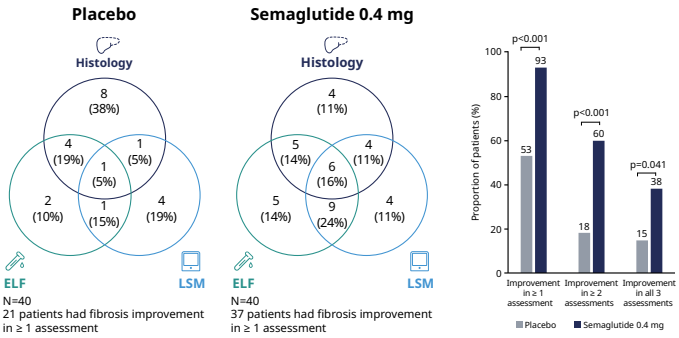
Wholistic Assessment of Biomarker Response

Overview of bi-directional NIT changes per study arms



Assessment of consistency of NIT change at the *per patient* level



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1. Anstee et al, Fibrosis response assessed by enhanced liver fibrosis and FibroScan liver stiffness measurement in patients with non-alcoholic steatohepatitis treated with subcutaneous semaglutide. EASL 2021.

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Meta-Thoughts on Response Biomarkers in NASH Drug Development

1. Rapidly attaining a NIT 'response threshold' is not a guarantee of early efficacy.
2. Interpret changes in 'Simple Panels' (APRI, FIB4, NFS) with caution.
3. **In the absence of a single 'gold standard' biomarker, undertake a wholistic assessment of biomarker response and demonstrate change *at the patient level*.**
4. **We should agree and define a common set of biomarkers that will be measured *and reported* in all future clinical trials.**

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Conclusions

- **A range of tractable biomarkers are available to support drug development in NASH.**
- When considering the use of biomarkers in drug development, it is essential to consider the specific **Context of Use** (and population/setting) that is being addressed.
- There remains a need for more sensitive and specific, independently validated and qualified biomarkers for use in NAFLD drug development.
- Progress to date:
  1. **Diagnostic CoU:** LITMUS and NIMBLE are bringing clarity and objectivity to biomarker performance – still room for innovation to identify better biomarkers.
  2. **Pharmacodynamic/Response CoU:** remains an area where there is a lack of consistency but the RCTs to date have helped generate important data to support biomarker utility.

     We are now better placed to build consensus and *standardise NIT selection* and the *consistency of NIT reporting* as study endpoints to support NASH drug development.
  3. **Surrogacy potential:** It is notable that there is an expanding dataset to demonstrate that fibrosis biomarkers have prognostic value and change with progression/regression of disease, potentially with greater inter-test consistency than histology.

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Acknowledgement

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quentin.anstee@newcastle.ac.uk

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